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Undifferentiated Sarcomas of Children: Pathology and Clinical Behavior—An Intergroup Rhabdomyosarcoma Study

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Undifferentiated soft tissue sarcoma (UND-STS) is the most poorly defined tumor eligible for Intergroup Rhabdomyosarcoma Studies (IRS). Recent IRS UND-STS experience was reviewed to assess the histologic characteristics and clinical behavior of undifferentiated sarcomas. Of the 1,527 patients entered on IRS-III and IRS pilot-IV, 96 had tumors classified by the IRS Pathology Committee as UND-STS. Of these, 52 had adequate histologic material for this study. After application of immunohistochemistry, 18 tumors were reclassified, mostly as embryonal rhabdomyosarcomas (RMS), primitive neuroectodermal tumors, and intra-abdominal desmoplastic small round cell tumors. The remaining 34 UND-STS had a diffuse hypercellular histologic pattern made up of sheets of medium-sized cells. The tumor cells had a minimal to moderate amount of cytoplasm and a variable nuclear morphology, predominately vesicular with finely granular chromatin. Except for reactivity with antibodies against vimentin, most tumors had a negative immunohistochemical profile. The 5 year Kaplan-Meier survival estimate for patients with non-metastatic disease was 72%, a significant improvement when contrasted with patients diagnosed to have UND-STS in IRS-I and IRS-II. Med. Pediatr. Oncol. 29:170–180, 1997.

Key words: undifferentiated soft tissue sarcoma; rhabdomyosarcoma; pediatric tumors; immunohistochemistry; survival

INTRODUCTION

Pediatric soft tissue sarcomas, largely characterized by primitive cellular elements, have long been problematic to diagnose and classify. With the Horn and Enterline classification it was demonstrated that prognostic significance could be attributed to the subtypes of embryonal and alveolar rhabdomyosarcoma (RMS), and tumors were diagnosed as RMS largely by inferring myogenesis from hematoxylin-eosin (H&E) staining characteristics [1–3]. Related neoplasms in which myogenesis could not be demonstrated were placed into a category labeled ''small round cell sarcoma, type indeterminate'' [1]. Some of these tumors were found to be morphologically indistinguishable from Ewing's sarcoma of bone, and a new category, ''extraosseous Ewing's sarcoma (EOE),'' was proposed [4–8]. It soon became apparent that considerable overlap existed between the EOE and the primitive neuroectodermal tumor (PNET) [5,9–15].

Despite the identification of the EOE/PNET subgroup, a significant group of tumors remained which could not be further classified. These tumors were composed of larger, more pleomorphic uncommitted cells, showing no evidence of myogenesis or other differentiation. Most of these tumors had a diffuse pattern without discernible architectural features, and were designated ''undifferentiated sarcomas'' [16]. In an analysis of 1,626 patients registered in Intergroup Rhabdomyosarcoma Studies (IRS)-I and IRS-II between 1972 and 1984, the diagnosis of undifferentiated soft tissue sarcoma (UND-STS) was made in 135, or 8% of patients [1].

With increased use of immunohistochemistry, it became clear that some lesions previously diagnosed as UND-STS could be given a more specific diagnosis. This study was designed to review all tumors classified as undifferentiated sarcoma in the IRS-III and IRS pilot-IV studies, with the 2-fold purposes of 1) ascertaining how immunohistochemistry and recognition of newly described pathologic entities (such as the intra-abdominal desmoplastic small round cell tumor and rhabdoid tumor of soft tissue) impact upon reclassifying these neoplasms,

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For the Intergroup Rhabdomyosarcoma Committee, representing the Children's Cancer Group (BRP, WAN, ABH, SJQ, FBR, HMM), and the Pediatric Oncology Group (HMM, BLW), the Pediatric Intergroup Statistical Center (LA).

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and 2) reviewing the histology and clinical behavior of the remaining undifferentiated sarcomas.

MATERIALS AND METHODS

Due to insufficient archival tissue for immunohistochemical analysis, cases from the earlier IRS-I and IRS-II were not included. This analysis was limited to IRS-III and IRS pilot-IV studies, in which a total of 1,527 patients were registered between November 1984 and February 1990, and followed up until January 1994.

Material submitted to the IRS Pathology Center consisted of stained and unstained tissue sections with pathology and operative reports. Patients were staged according to the IRS Clinical Grouping Classification. Clinical group I patients had complete removal of localized disease. Clinical group II patients had gross resection of their tumors with microscopic residual disease, with or without positive regional lymph nodes, or had complete resection of their tumors and resected positive lymph nodes without microscopic residual disease. Clinical group III patients had gross evidence of residual tumor following surgery or biopsy, and clinical group IV patients had metastatic disease at time of diagnosis. Most cases were obtained from excisional biopsy or tumor resection, although incisional biopsy specimens were accepted provided that adequate amounts of viable tumor were obtained.

All cases originally diagnosed as undifferentiated sarcoma were first reviewed for this study by one pathologist (B.R.P.), and then later by two IRS Pathology Committee reviewers (W.A.N. and A.B.H.). The first review was performed on H&E-stained tissue sections obtained from the IRS files. When the material available was suboptimal for histologic analysis, an effort was made to obtain interpretable histology by procuring the original paraffin blocks from the referring centers. Cases in which interpretable sections could not be obtained were excluded. Immunohistochemical analysis was performed utilizing a fixed battery of primary antibodies in all cases in which adequate tissue was available. This battery consisted of skeletal muscle specific actin (DAKO, Carpinteria, CA), desmin (DAKO), vimentin (DAKO), S-100 protein (DAKO), neuron specific enolase (DAKO), myoglobin (DAKO), leukocyte common antigen (DAKO), wide spectrum keratin (DAKO), and epithelial membrane antigen (DAKO). When tissue was limited, differential diagnostic impressions based on H&E morphology directed the choice of antibodies used. Adequate material was available to apply 7 or more immunohistochemical stains in 29 cases. Pepsin (Sigma, St. Louis, MO), 10% in 1 N HCl, was used for tissue digestion before use of the primary antiserum (45 min at 37°C). The avidin-biotin amplification system was employed in the immunostaining procedure, and the reaction was developed with 3-amino-9-ethylcarbazole (0.2 mg/ml in 0.02 M sodium acetate buffer, pH 5.0). Microwave retrieval was not utilized. Mayer's hematoxylin was used as counterstain.

TABLE I. Diagnoses of 18 Reclassified Tumors

Revised diagnosis	No. of cases
Embryonal RMS	5
PNET	5
Intra-abdominal desmoplastic small cell tumor	3
Synovial sarcoma	1
Malignant fibrous histiocytoma	1
Infantile fibrosarcoma	1
Lymphoma	1
Alveolar RMS	1

In addition to the standard battery, the following antisera were used in selected cases: 12E7 (furnished by Dr. Michael Link, Stanford University, Stanford, CA), HBA-71 (furnished by Dr. Gerhard Hamilton, University School of Medicine, Vienna, Austria), neurofilaments (DAKO), glial fibrillary acidic protein (DAKO), lysozyme (DAKO), chromogranin (DAKO), alpha fetoprotein (DAKO), alpha-1-antitrypsin (DAKO), smooth muscle specific actin (DAKO), and factor VIII antigen (DAKO).

Mitotic activity and degree of cytologic atypia were graded, based upon a modification of the soft tissue sarcoma grading criteria of Coindre et al. [17]. Mitotic activity was defined as high for tumors with greater than 10 mitoses per 10 high power fields and low for tumors with less than 10 mitoses per 10 high power fields. Degree of atypia was defined as minimal (generally round to oval or spindle shaped nuclei with finely dispersed chromatin), moderate (enlarged, hyperchromatic nuclei, often with prominent nucleoli), or marked (giant tumor cells and/or bizarre nuclear shapes).

The data base from the Intergroup Statistical Center was utilized for statistical analyses. Survival time (S) was measured as the time from start of treatment to death for patients who have died and the time to latest follow-up for all patients still alive. Curves plotting the distribution of survival time were calculated by the method of Kaplan and Meier, and differences among distributions were tested using the Gehan-Breslow tests [18,19]. $P \leqslant 0.05$ was considered highly statistically significant and strong statistical evidence against the null hypothesis.

RESULTS

Of the 1,527 patients in the IRS-III and IRS pilot-IV studies, 96 were registered as UND-STS; 52 of these had adequate clinical data and tissue available for study. Histologic and immunohistochemical analysis led to the reclassification of 18 of these cases (Table I). Six (33%) were diagnosed as RMS: 5 embryonal and 1 alveolar subtype. Five (28%) were diagnosed as PNET and 3

TABLE II. Clinicopathologic Features of Undifferentiated Sarcomas*

Case	Site	Age (years)	Sex	Size (cm)	Clinical group	Histologic type	Mitotic activity	Atypia
1	Leg	12	M	5	I	Dif	H	2
2	Shoulder	2	F	6	I	Nes	L	2
3	Abdominal wall	16	F	5	II	Dif	H	2
4	Shoulder	0	M	5	I	Spi	L	2
5	Chest	15	F	2	I	Nes	H	2
6	Thigh	10	M	5	I	Dif	H	2
7	Thigh	3	M	6	I	Dif	H	2
8	Neck	16	F	11	II	Nes	H	2
9	Scalp	4	M	3	III	Des	H	2
10	Foot	5	M	3	II	Dif	H	3
11	Shoulder	20	F	9	II	Des	L	2
12	Chest	10	F	19	II	Dif	L	1
13	Retroperitoneum	3	F	7	III	Dif	H	2
14	Buttock	7	M	3	III	Spi	H	1
15	Pharynx	6	M	5	III	Spi	H	2
16	Abdominal wall	15	F	7	IV	Dif	H	3
17	Neck	18	M	5	IV	Dif	L	2
18	Chest	14	M	8	III	Dif	L	3
19	Back	15	M	5	III	Des	H	2
20	Forearm	12	F	20	III	Spi	H	2
21	Shoulder	4	M	8	III	Nes	L	2
22	Buttock	0	M	10	IV	Spi	H	1
23	Thigh	10	M	15	III	Dif	H	2
24	Chest	3	M	9	III	Dif	H	3
25	Buttock	12	M	14	IV	Dif	H	2
26	Leg	6	M	ND	IV	Dif	L	1
27	Thigh	14	F	9	III	Nes	L	1
28	Vagina	1	F	ND	III	Dif	L	1
29	Leg	13	M	10	III	Dif	H	2
30	Forearm	0	F	6	III	Dif	H	1
31	Chest	0	M	7	III	Nes	H	2
32	Neck	0	M	4	IV	Dif	H	3
33	Leg	15	M	12	IV	Dif	H	2
34	Neck	10	F	7	IV	Dif	H	2

*Dif, diffuse; Nes, nested; Spi, spindle; Des, desmoplastic; H, high; L, low; ND, not done. Degree of atypia: 1, minimal; 2, moderate; 3, marked.

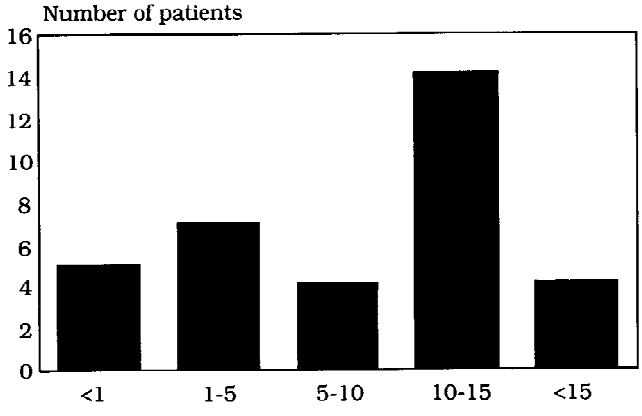

Fig. 1. Distribution of ages at diagnosis of the 34 children and adolescents with undifferentiated sarcoma.

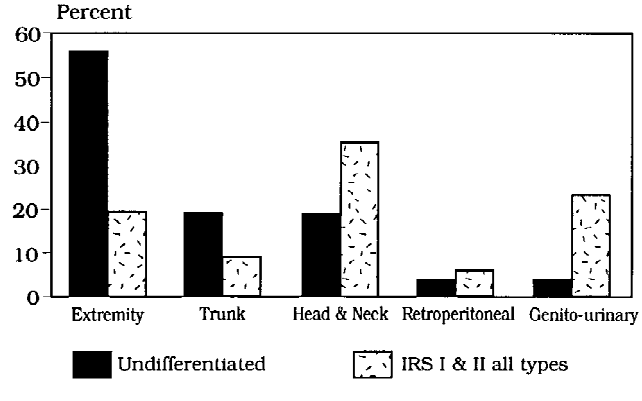

Fig. 2. Distribution of sites of primary tumors in 34 children and adolescents with undifferentiated sarcoma compared to the sites in all 1,626 eligible patients in IRS-I and IRS-II combined.

(17%) as intra-abdominal desmoplastic small round cell tumors (IADSRCT).

The RMS were composed of diffuse sheets of densely packed primitive small cells with scanty amounts of cytoplasm and unconvincing evidence of myogenesis by routine staining. Their nuclei were hyperchromatic and nucleoli were inconspicuous. One lesion had a prominent spindle cell pattern. All showed crisp cytoplasmic stain-

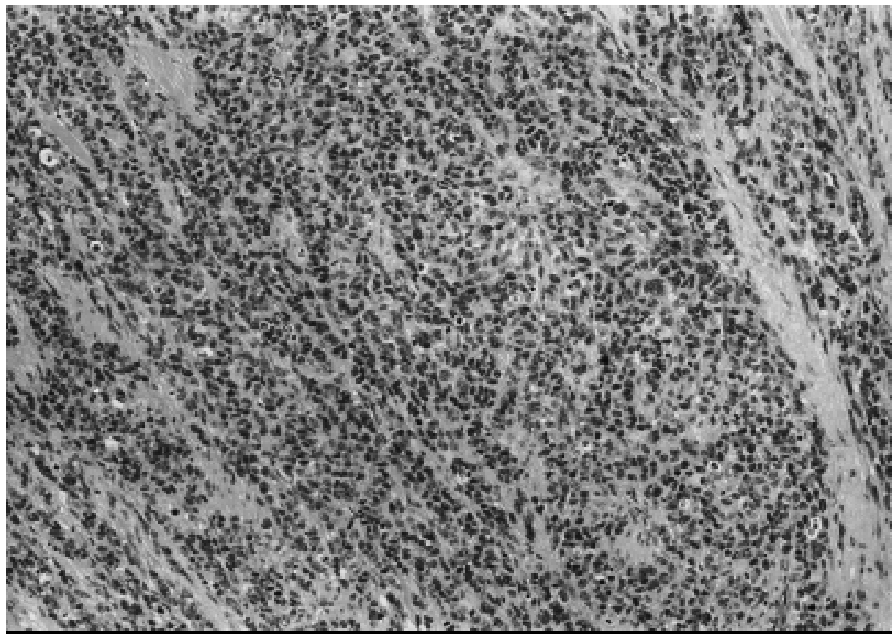

Fig. 3. Diffuse pattern of undifferentiated sarcoma. There are sheets of medium-sized cells with poorly defined cytoplasm and vesicular nuclei (H&E stain; ×100).

ing for skeletal muscle specific actin (5/6) and/or desmin (4/6). These were seen in patients with a mean age of 4.3 years (range: 6 months–8 years). There were 2 males and 3 females. The tumors ranged from 4 to 8 cm in maximum dimension (mean: 6 cm); 2 were located in the head and neck, 2 in the pelvis, and 1 in the upper extremity.

Anti-MIC2 antibodies were applied to 6 tumors with histology suggestive of PNET. These were characterized by sheets of monotonous cells which tended to be smaller than the other UND-STS, hyperchromatic nuclei, minimal cytoplasm, and inconspicuous nucleoli. All but one of these tumors were positive for one or both of the anti-MIC antibodies, HBA-71 and 12E7, and negative for other immunohistochemical markers except for vimentin and neuron specific enolase (positive in 2/5). The 5 tumors with positive anti-MIC staining were therefore reclassified as PNET on the combined basis of histologic and immunohistochemical parameters. Unfortunately, cytogenetic and molecular data were not available for these archival specimens. The patients with PNET spanned an age range of 2–18 years (mean: 11 years); 4 were female and 1 was male. Tumors ranged in size from 2 to 20 cm (mean: 11.3 cm), and were located in the chest (3 cases) or lower extremity (2 cases).

Histologically, the 3 cases of IADSRCT showed sheets of tumor cells with a diffusely infiltrative pattern, separated into nests by dense collagenous stroma. The tumor cells were undifferentiated and small, with fairly inapparent cytoplasm and round to oval nuclei. Immunohistochemical analysis performed with the battery of 9 antibodies showed 3 patterns of positive staining: desmin (Des), vimentin (Vim), keratin (Ker), epithelial membrane antigen (EMA); Des, Vim, Ker; and Des, neuron specific enolase (NSE). The patients' ages ranged from 8 to 15 years (mean: 11 years); all were male. The tumors were located in the abdomen (1 case) or pelvis (2 cases), and had diameters ranging from 8 to 24 cm (mean: 13 cm).

A total of 34 cases of UND-STS remained following review and reclassification. The clinicopathologic features are outlined in Table II. Ages ranged from newborn to 20 years, with a mean of 9 years. Most (65%) were older than 5 years, similar to the IRS-I-II distribution of patients with alveolar RMS, but in sharp contrast to those with embryonal RMS (Fig. 1). There were more boys (62%) than girls, and the most common site was in an extremity (53%) (Fig. 2). Of the 18 extremity tumors, 9 were located in the lower limb and 6 in the upper limb, including the should girdle. The remaining 3 originated in the buttocks. Five were present in the chest wall, 4 in the neck, 2 in the abdominal wall, and 1 was present in each of the following sites: scalp, retroperitoneum, pharynx, back, and vagina. More than half of the tumors (59%) measured greater than 5 cm. Forty-four percent of the patients were in clinical group (CG) III, followed by CG IV (24%), CG I (18%), and CG II (15%).

Histology of Undifferentiated Sarcomas

Most cases (59%) were composed of diffuse sheets of medium-sized packed cells with no discernible architectural structure. Cellularity tended to be high, with minimal interstitial ground substance (Fig. 3). Necrosis and inflammation were not prominent. The cells had inappar-

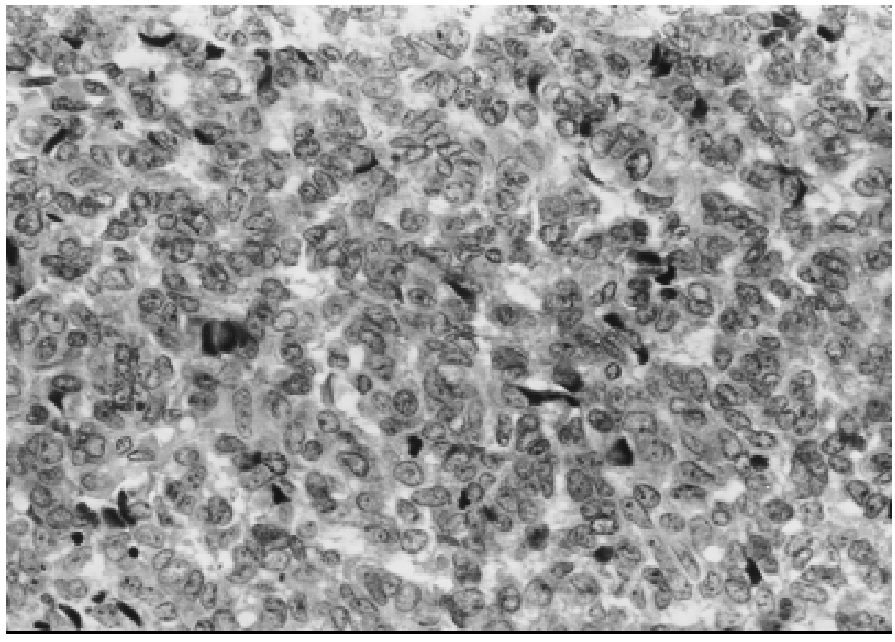

Fig. 4. Diffuse pattern. Fascicles of polygonal cells are present in a background of moderate eosinophilic amorphous ground substance. Cells have oval to angulated nuclei with prominent chromocenters. Several cells are apoptotic (H&E stain; ×400).

ent to moderate amounts of cytoplasm and poorly defined cell borders (Fig. 4). Nuclear structure was heterogeneous. Many nuclei were vesicular with numerous chromocenters; others were remarkable for the prominence of their nucleoli (Fig. 5). Most cases had high mitotic activity, with greater than 10 mitoses per 10 high power fields. Atypia was graded as moderate in two thirds, with the remainder divided nearly evenly between minimal and marked.

In 6 cases, the featureless diffuse pattern was modified by the presence of delicate fibrovascular septae, subdividing the neoplasms into nests, reminiscent of hemangiopericytoma (Fig. 6). These tumors had similar cytologic characteristics to other UND-STS.

Five lesions had a spindle to vague storiform pattern, with the neoplastic cells arranged in poorly defined fascicles (Fig. 7). Three were remarkable for the presence of broad bands of collagen (Fig. 8). The cytologic features of both the spindle and desmoplastic tumors were similar to the other UND-STS, with predominantly oval nuclei, prominent chromocenters, and indistinct cytoplasm.

Immunohistochemistry of Undifferentiated Sarcomas

Immunohistochemical results are given in Table III. With the exception of vimentin (77% positivity), these tumors were generally non-reactive to the panel of 9 antibodies. All failed to react with antibodies against actin, desmin, and leukocyte common antigen. Five tumors showed reactivity with either keratin or epithelial membrane antigen, and 3 reacted with antibodies directed against S-100 protein. One had a positive reaction against myoglobin. Due to its repeated non-reactivity for actin and desmin and atypical cytology (large, polygonal cells), it was felt that insufficient evidence was present to justify reclassification to RMS.

Survival

There was statistical evidence of differing survival ($P = 0.01$) by clinical group for patients whose lesions were reclassified as undifferentiated sarcoma (Fig. 9). The estimated survival at 5 years was 25% (SE 15.3%) for patients with metastatic disease and 73% (SE 8.4%) for patients with non-metastatic disease. Among the 26 non-metastatic patients, tumor size ($<$ or $\geqslant 5$ cm) and regional lymph nodes did not prove to be statistically significant ($P = 0.482$ and $P = 0.120$, respectively), which could be due to the small number of patients in our study.

The estimated survival rates for patients whose tumors occurred in sites identified in previous IRS studies as being unfavorable (parameningeal, extremity, trunk, and retroperitoneal) are illustrated in Figure 10. There was a statistically significant difference in survival experience by primary site ($P = 0.05$). At 5 years, approximately 92% (SE 7.3%) of the patients with non-metastatic extremity tumors had survived compared to 58% (SE 16.1%) for the rest of the non-metastatic unfavorable sites.

DISCUSSION

Soft tissue tumors of childhood present a unique diagnostic challenge. The primitive nature of these neo-

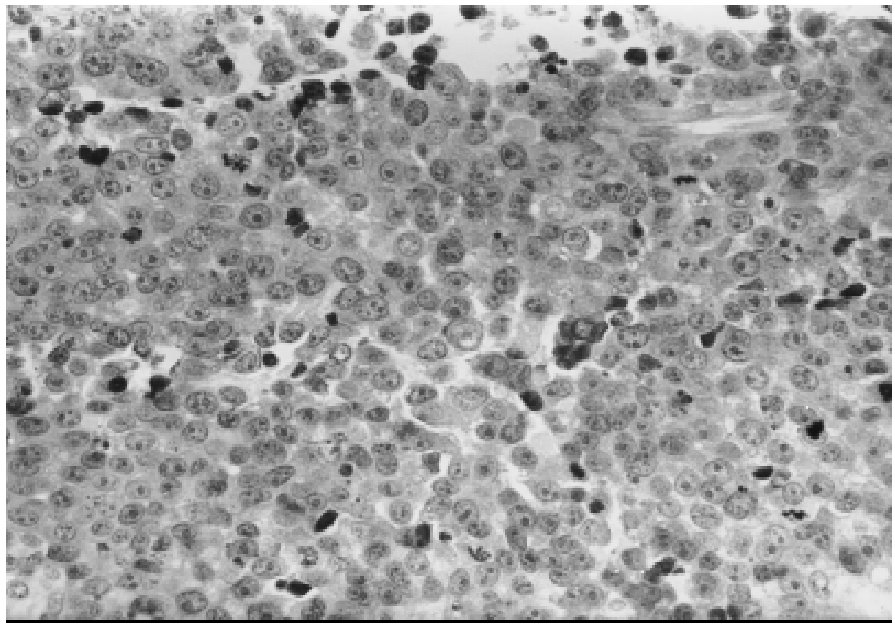

Fig. 5. Diffuse pattern of undifferentiated sarcoma showing cells with prominent nucleoli (H&E stain; ×400).

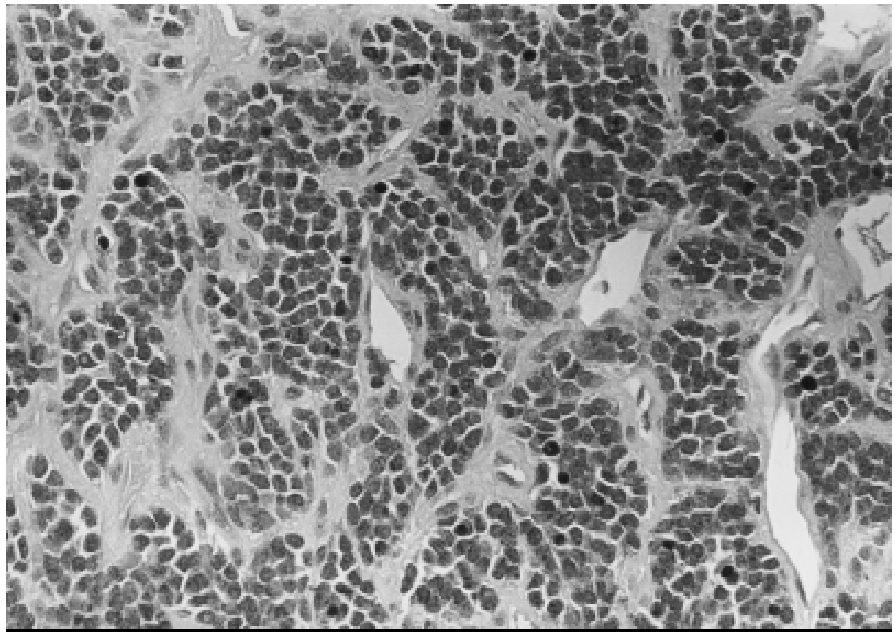

Fig. 6. Nested pattern of undifferentiated sarcoma. Nests of cells are separated by a delicate fibrovascular stroma (H&E stain; ×100).

plasms and subtle histologic differences between subtypes often make accurate diagnosis difficult. This problem has been aggravated by their rarity, precluding any one center from seeing many cases. Classification of these tumors has traditionally been based upon either identifiable tissue specific histogenesis (e.g., fibrosarcoma, RMS) or unique histologic patterns repetitive enough to warrant a specific diagnosis even though the tissue of origin is not clear (e.g., alveolar soft part sarcoma, epithelioid sarcoma, rhabdoid tumor). Soft tissue tumors of children characteristically consist of immature cells, which sometimes have been given a specific designation even if only a few cells demonstrate evidence of a specific cell type of origin. While technical limitations, inadequate preservation, or small amounts of tissue for study account for some of the difficulties, even with optimum tissue and the most careful application of the most modern technology, some tumors lack specificity and continue to defy classification. In general, it has been estimated that about 10% of soft tissue tumors cannot be

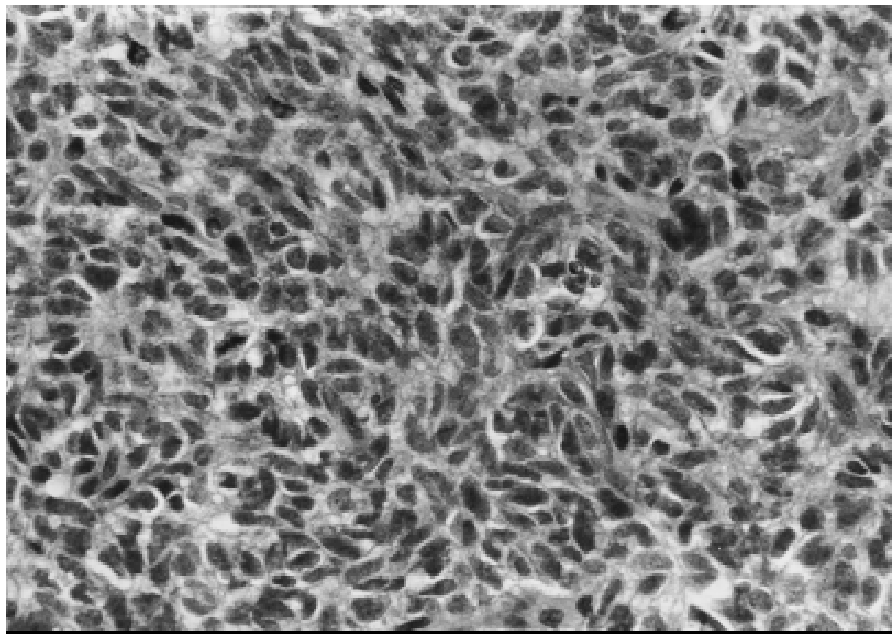

Fig. 7. Spindled variant of undifferentiated sarcoma. There is a vaguely storiform pattern (H&E stain; ×400).

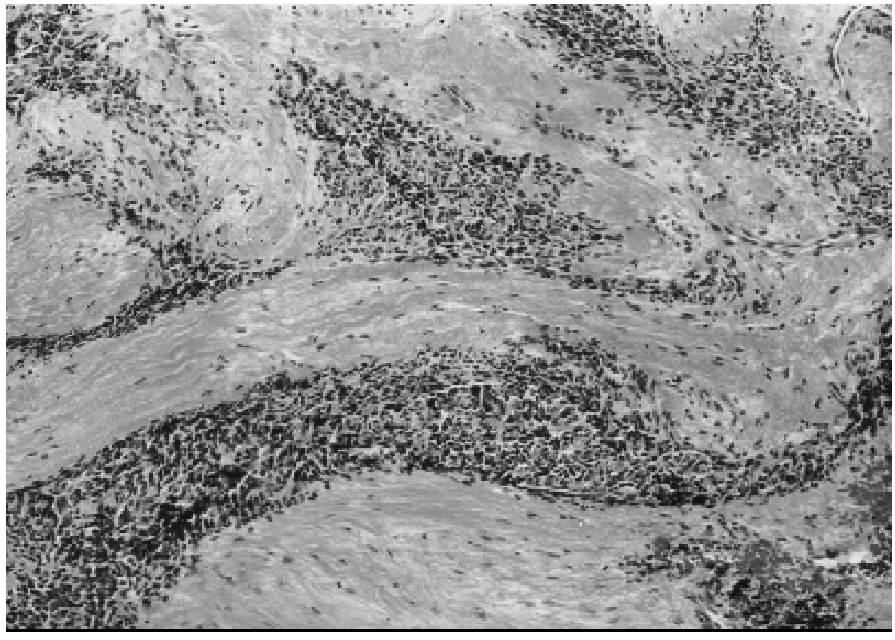

Fig. 8. Desmoplastic variant of undifferentiated sarcoma (H&E stain; ×100).

placed into a definite diagnostic category irrespective of the diagnostic experience and knowledge of examining pathologists [20]. The large proportion (46%) of cases of UND-STS excluded from this study perhaps underscores this difficulty, as the most common reason for exclusion was inadequate tissue remaining in the block for additional immunohistochemistry.

Historically, Anderson's [21] survey of childhood cancers included 24 soft tissue tumors, of which she considered half to be of doubtful diagnosis. Soule et al. [22] emphasized this group of undifferentiated tumors in their clinicopathologic study of 135 cases seen at the Mayo Clinic from 1950 through 1965. A fifth of their lesions were not classifiable to a specific cell type and were designated sarcomas of undetermined histogenesis. Others have commented on this in reviews of childhood sarcomas, including the series of cases treated in the early Children's Cancer Study Group protocols [16]. In IRS-I and IRS-II, in which both RMS and sarcomas of undetermined histogenesis of children and adolescents

TABLE III. Immunohistochemical Results of Undifferentiated Sarcomas*

Case	ACT	DES	VIM	S-100	NSE	MYO	LCA	KER	EMA
1	−	−	+	−	−	−	−	−	+
2	−	−	+	−	−	−	−	−	−
3	I	−	+	−	I	ND	−	−	ND
4	−	−	+	+	I	I	−	−	−
5	−	−	−	I	−	−	−	−	−
6	−	I	+	I	−	I	I	−	−
7	−	I	ND	−	ND	−	ND	ND	ND
8	−	−	−	−	−	−	−	−	−
9	−	−	−	−	−	−	−	−	−
10	−	I	+	I	−	−	−	−	−
11	−	−	−	−	−	−	−	−	−
12	−	−	+	−	−	−	−	ND	−
13	−	I	+	−	−	−	−	−	−
14	I	−	+	−	−	−	−	−	−
15	−	−	ND	+	−	−	ND	+	ND
16	I	I	+	−	ND	I	−	−	−
17	−	−	+	−	−	I	−	+	−
18	−	−	+	−	−	−	−	−	−
19	−	−	−	−	−	ND	−	ND	−
20	I	I	I	+	−	ND	ND	−	−
21	−	−	+	−	−	I	−	I	+
22	−	−	+	I	−	−	ND	−	−
23	−	I	+	−	I	−	−	I	+
24	ND	−	+	−	−	ND	ND	−	ND
25	−	−	ND	−	−	−	ND	−	−
26	ND	−	+	−	ND	−	−	−	−
27	−	−	I	−	−	ND	−	−	−
28	−	−	+	ND	−	I	−	−	−
29									
30	−	−	+	−	−	−	−	−	ND
31	−	−	+	I	−	ND	ND	−	−
32	ND	−	+	ND	−	I	−	−	−
33	−	−	+	−	−	+	−	−	ND
34	−	I	+	−	−	−	−	−	−

*ACT, actin; DES, desmin; VIM, vimentin; NSE, neuron specific enolase; MYO, myoglobin; LCA, leukocyte common antigen; KER, keratin; EMA, epithelial membrane antigen. I, indeterminant staining; ND, not done.

were study eligible, review of 1,626 lesions showed that 135 (8%) could not be specifically classified and were considered undifferentiated sarcomas [1,23–25]. Application of currently available immunohistochemistry procedures, together with classic ultrastructure analysis, has permitted a reduction in the number of lesions which cannot be classified [26,27]. The recent recognition of the group of tumors now designated as PNET, IADSRCT, and ''solid'' alveolar RMS have further reduced the number of these previously ill-defined primitive tumors [28–31]. However, there still remains a small group of about 5% of soft tissue tumors of children that are best classified as undifferentiated sarcoma. In contrast to a reasonable level of reproducibility between the referring institution and the IRS review committee for both embryonal and alveolar RMS, there has been only a 68% concordance for UND-STS, reflecting their poorly defined nature [1]. This study was prompted by the opportunity to systematically apply immunohistochemical diagnostic techniques to a reasonable number of this group of neoplasms.

In this study, 35% of cases initially diagnosed as UND-STS were reclassified. Embryonal RMS, PNET, and IADSRCT were the most common tumors to be confused with UND-STS. Although the typical embryonal RMS showing histologic evidence of myogenesis would not be expected to cause diagnostic trouble, poorly differentiated cases exist in which myogenesis can only be demonstrated with the use of skeletal muscle specific actin and desmin, the most useful immunohistochemical markers for RMS. Although these markers may be present in unrelated pathologic conditions, a current study of over 100 IRS-IV cases has shown polyclonal desmin to have a sensitivity of over 98%, and anti-skeletal muscle actin a sensitivity of over 94% in RMS [3] (Hojo, personal communication). In this study, tumors that were histologically compatible with RMS and positive for one or both muscle markers were considered RMS and not UND-STS.

The anti-MIC2 antibodies 12E7 and HBA-71 were found to be extremely helpful in evaluating neoplasms in which PNET/EOE was a diagnostic consideration. These

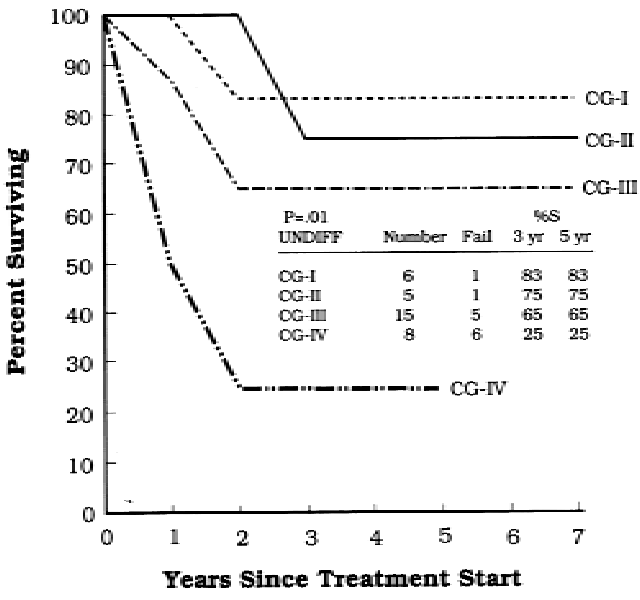

Fig. 9. Kaplan-Meier survival estimates by clinical groups for patients with undifferentiated sarcoma. CG = IRS clinical group; UNDIFF = undifferentiated; S = surviving.

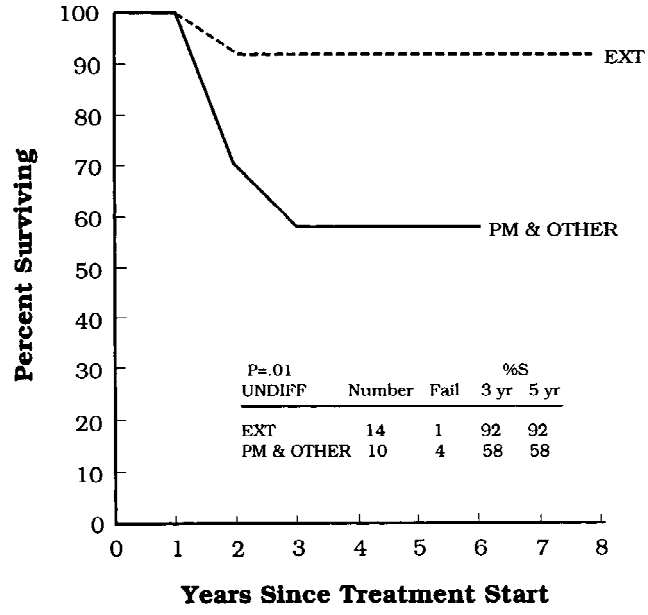

Fig. 10. Kaplan-Meier survival estimates by anatomic site for nonmetastatic patients with undifferentiated sarcoma. UNDIFF = undifferentiated; PM = parameningeal; OTHER = sites other than extremity, head and neck, and non-bladder-prostate genitourinary sites; EXT = extremity; S = surviving.

antibodies have been shown to have a high degree of sensitivity and specificity for this family of tumors, especially when used in conjunction with leukocyte common antigen and immunohistochemical muscle markers to rule out a hematopoietic neoplasm or RMS [32,33]. Positive staining for one or both of these MIC2 antigens with other negative markers was a key element in making the diagnosis in all 5 cases of PNET/EOE. Immunohistochemistry was also useful in confirming the diagnosis in the 3 cases of IADSRCT [28].

In the first two IRS series, 8% of cases were diagnosed as UND-STS. In the current study, 65% of cases initially diagnosed as UND-STS retained this diagnosis. Since 96 of the 1,527 cases accessioned in the IRS-III and IRS pilot-IV studies were initially diagnosed as UND-STS, it is estimated that if adequate tissue were available in all cases, approximately 63 of 1,527 cases (0.65 × 96) would retain the diagnosis, accounting for 4% of pediatric RMS and allied tumors. This reduction in frequency can be attributed to the application of immunohistochemistry and the recognition of the tumors described above.

Even with a limited number of cases, several features of UND-STS appear noteworthy. These tumors have a site predilection: 53% of UND-STS in this study occurred in an extremity, as opposed to 18% of all cases accessioned in IRS-I–III. Also, UND-STS are more likely to occur in a truncal location (23%), as opposed to 8% in IRS-I–III [1]. Although these sites have been associated with a poor prognosis in previous IRS studies, this did not hold in this study. Especially noteworthy was the 5 year survival rate for non-metastatic extremity tumors of 92%.

The overall 5 year survival for our 34 cases was 62%, with 73% survival at 5 years for non-metastatic UND-STS. This contrasts with the 47% overall survival of UND-STS found in a recent analysis of IRS-II data [3]. Although the reasons for this difference are not entirely clear, our study suggests that the better than expected survival may be at least partly due to the reclassification of a significant percentage of cases originally diagnosed as UND-STS, with many falling into more unfavorable prognostic categories. More than half of tumors were ⩾5 cm in diameter, and 24% were associated with metastases at diagnosis.

The improvement in survival for patients with UND-STS in this review was most notable in CG I and II with no residual and microscopic residual, respectively. Unlike patients with alveolar RMS, who were selectively assigned more intensive chemotherapy following pretreatment histologic review, patients in IRS-III with CG I and II UND-STS and more favorable embryonal RMS received similar radiotherapy and chemotherapy which was not dramatically different from IRS-II. The surgical guidelines for IRS-III, however, strongly advocated planned re-excision (PRE) of the primary tumor site [34]. Trunk and extremity sites were the major focus for PRE and comprised 76% of the sites for UND-STS in this series. The initial utilization of a more assiduous surgical strategy, PRE, in the context of a comprehensive protocol appears to have improved the survival of patients with UND-STS who thereby entered into further multidisciplinary treatment with minimal residual disease

[34]. Since only 8 patients who were treated on 6 different chemotherapy regimens had metastatic UND-STS, comparisons of improved survival are very tenuous.

This study did not find these tumors to have any histologic features which could be used for prognostically meaningful subclassification. Although a minority of tumors had a variable amount of supporting stroma, most were relatively featureless and diffuse. The variability of cytologic and nuclear features would indicate that these are heterogeneous neoplasms.

Although invaluable in separating out poorly differentiated related specific tumors, immunohistochemistry usually has, with the exception of vimentin, a negative profile in UND-STS. Sporadic positive staining for epithelial markers and S-100 protein, unsupported by other signs of differentiation, is inconclusive, but could reflect a heterogeneous histogenetic nature. Despite immunostaining for epithelial markers in these few UND-STS, these tumors did not have the morphology of synovial or epithelioid sarcomas.

Acknowledging that the immunohistochemical characteristics of RMS and related tumors are highly complex and that some tumors may display an unexpected or aberrant immunophenotype, it could be argued that too heavy a reliance has been placed on immunohistochemistry in this study. Within the limitations of the use of archival material, immunohistochemistry in conjunction with careful histologic assessment was felt to be the most practical tool available for retrospective study. Electron microscopy was not referred to due to the lack of uniformity in the application of this technique at local institutions.

In order to better understand these neoplasms, a multimodality prospective approach needs to be applied. While many of the cases now diagnosed as UND-STS may represent tumors too primitive to be characterized by routine histology, electron microscopy and immunohistochemistry, other methods may prove to be fruitful in defining the histogenesis of these tumors. Cytogenetic analysis for chromosomal abnormalities, fluorescence in-situ hybridization, DNA ploidy, and molecular pathologic investigation for MyoD1 protein expression and N-Myc gene amplification are all modalities which are showing promise for future investigation [35-38]. It is hoped that with judicious application of these techniques, the number of pediatric sarcomas which defy further characterization will continue to diminish.

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